

Genetics and Tropical Forests

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Abstract

Trees compared to other organisms have a very high level of genetic diversity (Hamrick and Godt 1990). As sessile long-living plants, they need this high level of genetic variation for adaptation to highly variable environmental conditions. A set of population genetic processes such as the mating system, gene flow, selection, and migration determine the genetic composition of a tree population. First we will give an introduction and overview on the most important processes and their impact on the genetic diversity of tropical trees, and then we will go in more details for some of the processes. The genetic diversity is of fundamental function for the stability of forest ecosystems. Thus adequate measures on gene conservation are very important. We will cover this topic with a specific chapter. More recently with the advance of genomics and molecular genetics, we have a broad set of molecular markers in hand for diagnostic purposes. We highlight as one important application the use of gene markers and DNA bar coding for tree species identification and as a tool to fight against illegal logging. Finally we will give an introduction to tree breeding programs and provenance tests in the tropics.

Keywords

Animal Dispersal; Forest Fragmentation; Genetic Conservation; Gene Flow; Mating System; Pollen Dispersal; Seed Dispersal; Spatial Genetic Structure; Tree Breeding; Tropical Trees.

Introduction and Overview on Factors Determining the Genetic Composition of Tropical Trees

Older publications assumed that tropical tree populations have only a limited genetic diversity and a high level of inbreeding because of the low densities of trees of the same species, the non-synchronized flowering, and the sensitive dependence on animals as vectors for pollen and seed dispersal (Corner 1954; Federov 1966). But this assumption could be rejected first with allozyme studies and later with results on molecular gene markers. In fact tropical tree populations have a similar level of genetic diversity compared to tree species in the temperate zones, and most of the tropical tree species are outcrossers and have no or only a limited level of inbreeding (Loveless 1992, 2002; Murawski and Hamrick 1991).

An important reason for the comparable high level of genetic diversity of tropical trees is their large reproductive neighborhood area. The densities of tropical conspecific trees are in most cases very low, but because of a very efficient pollen dispersal, trees of a large area are in reproductive

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contact (Dick 2001; Gribel et al. 1999; Nason et al. 1996; Stacy et al. 1996). Many of the pollinating animals especially bees, birds, and bats are capable to pollinate over very large distances up to a couple of kilometers (Roubik 2000). One of the largest distances for pollen dispersal has been found for *Ficus*. For this genus trees have been observed to be pollinated on more than 12 km (Nason et al. 1998). The respective neighborhood area covered more than 600 km².

Many tropical tree species have an asymmetrical pattern of gene flow by pollen and seeds. In most cases is the pollen dispersal much larger than the seed dispersal (Raspe et al. 2000; Hardy et al. 2004). Often animals such as monkeys, birds, bats, and rodents serve also as vectors for seed dispersal in the tropics. Especially bats are capable to transport seeds of up to dozens of km (Shilton et al. 1999). The dispersal of seeds by wind leads more to a regular distribution of seeds, but the distribution of seeds by animals is often clumped and aggregated (Julliot 1994). The distribution pattern of seeds is the most important factor creating a spatial genetic structure of trees in the tropics. This spatial genetic structure (SGS) with trees closer to each other being genetically more similar can have an extension of up to a few hundreds of meters (Caron et al. 2000; Degen et al. 2001; Degen and Roubik 2004). Another factor with a strong impact on the SGS is the aggregated distribution pattern of many tropical tree species (Caron et al. 2000; Degen et al. 2001; Dutech et al. 2002). Trees of the same species occur often in patches and among patches there are larger distances. Often an exchange of pollen among the patches is possible, but the seeds remain mostly within the same aggregate.

Many tropical tree species have efficient mechanisms to avoid selfing and mating among relatives. In the tropics we have a high proportion of dioeciously tree species with trees having either female or male flowers, and many species have genetically controlled incomparability systems (Bawa and Opler 1975). Most of the tropical tree species with male and female flowers on the same trees (co-sexual trees) have outcrossing rates higher than 85 % (Loveless 2002).

The genetic differentiation among tropical tree populations has been determined largely by the history of extinction and recolonization. The glacial periods changed the vegetation in the tropical regions a lot. During the peaks of the glacial periods, the area of tropical rain forests was drastically reduced, and former tropical rain forests have become a savannah-like dry vegetation (Colinvaux et al. 2000). When the climate got warmer again, the tropical rain forests remigrated from the refugia back into the former distribution areas. Depending on the refugia of origin, the trees have different genetic footprints that are especially visible in chloroplast genomes (Caron et al. 2000; Cavers and Lowe 2002; Dutech et al. 2000; Hardy et al. 2013). In Southeast Asia the glacial periods changed the seawater level a lot. At times with lower seawater level, the area of forest got even larger, and former islands got connected which had a significant impact on the genetic composition (Cannon et al. 2009). Independent of vegetation changes during the glacial periods, high mountains were always very efficient barriers to anticipate gene exchange. The Andes have been found to be such an efficient genetic barrier for many tree species on both sides of the mountain range (Cavers and Lowe 2002).

The fast deforestation in many regions of tropical forests is a serious problem for the genetic diversity of the tree populations. Only the Brazilian Amazon has an area of more than 3.5 million km². Every year 1 % and more of this area gets lost by deforestation (Broadbent et al. 2008; Nepstad et al. 1999). In the Neotropics humans have influenced and changed the forest composition even before the arrival of the European colonists. Especially in Central America it is reported that the natives have transferred former tropical forest to agricultural land (Leigh 1999). Selective logging instead of clear cuts is proposed as an alternative way to use and maintain tropical forests. In the last 15 years, several studies on the genetic impact of different intensities of selective logging on the genetic diversity of tree populations have been conducted (Obayashi et al. 2002; Degen et al. 2006; Sebbenn et al. 2008). Long-term field observations with repeated genetic inventories as well as

simulation studies have been done for this. The results are quite different depending on the life history traits of the tree species. In several cases it could be shown that the applied logging rules are not sustainable for neither the demographic structure nor the genetic diversity (Riina et al. 2014). There is an important interaction among the logging intensities and the overall change of forest composition on the landscape scale. Thus selective logging has a much stronger impact on genetic diversities of trees when it goes along with an increasing fragmentation of the surrounding landscape (Riina et al. 2014).

Gene Flow

Gene flow is the movement of genes among populations of a species. In tropical trees, as well as in other plants, gene flow occurs through pollen and seed dispersal. The gene flow is responsible for maintaining the genetic diversity of the populations (Robledo-Arnuncio and Gil 2005; Fuchs and Hamrick 2011). High rates of gene flow among populations reduce the genetic divergence among populations and low rates increase the genetic differentiation. Studies of gene flow and dispersal distances and general patterns of pollen and seed distribution are important to predict the future dynamics of populations and to develop strategies for gene flow (Austerlitz and Smouse 2001). Gene flow studies are also very useful to design efficient seed orchards for the production of improved forest reproductive material. In the case of natural and fragmented populations, it is desirable to have high rates of gene flow, since it increases the genetic diversity and effective size within populations (Burczyk et al. 2004; Bittencourt and Sebbenn 2007). In contrast for seed orchards, we would like to minimize the immigration of alien alleles via pollen because a contamination with outside pollen would reduce the genetic gain as a result of artificial selection of mating partners.

Pollen dispersal is a component of the mating system and is influenced by population density, flower phenology, vectors of dispersal, mechanism that reduces the selfing rate, and also by factors such as the climate, forest fragmentation, and logging. The main vector of pollen dispersal in most temperate tree species is the wind, and most tropical tree species are pollinated by animals. Seeds can be dispersed in both temperate and tropical trees by wind and animals, and in some cases water serves as vector of seed dispersal. Species pollinated by wind generally have lower genetic differences among populations compared to species pollinated by animals.

For animal-pollinated tree species, the type of pollinator partly determines the distances of pollen dispersal (Dick et al. 2008). And pollen dispersal is also affected by the population density, because at low densities, individuals are more widely dispersed and the pollinator needs to invest more energy to fly among co-flowering trees. Pollen dispersal distances are generally higher in low density compared to high-density populations (Ward et al. 2005; Dick et al. 2008). We found a significant negative Spearman ranking correlation between population density and average pollen dispersal distance (Table 1, Cor = -0.705 ($P < 0.05$)). Another effect of a low reproductive population size is an increase of mating among relatives. It can be measured as the proportion of correlated mating and coancestry within open-pollinated families (Aldrich and Hamrick 1998; Hanson et al. 2008). The average pollen dispersal distance rarely exceeds 300 m in forests with population density of >5 trees/ha (Table 1). A high frequency of short pollen dispersal distance combined with a low frequency of long pollen dispersal distance has been observed for several tree species (Geng et al. 2008; Lacerda et al. 2008; Silva et al. 2008; Jones and Muller-Landau 2008; Carneiro et al. 2009), suggesting that it is probably a general pattern of pollen dispersal for animal-pollinated species.

Table 1 A summary of genetic studies on pollen (realized and effective) flow: arithmetic mean, median, and maximum distance of pollen dispersal for tropical tree species

Tree species	Pollinator	Type of forest	Density (trees/ha)	External pollen flow (%)	Arithmetic mean (m)	Median (m)	Maximum (m)	References
<i>Araucaria angustifolia</i>	Wind	Fragmented	42.2	10	83	—	2,006	Bittencourt and Sebbenn (2007)
<i>Araucaria angustifolia</i>	Wind	Continuous	7.7	54	102	—	344	Bittencourt and Sebbenn (2008)
<i>Araucaria angustifolia</i>	Wind	Continuous	40.3	6	134	124	343	Sant'Anna et al. (2013)
<i>Carapa guianensis</i>	Butterflies	Continuous	5.6	30	195	181	430	Martins et al. (2012)
<i>Carapa guianensis</i>	Butterflies	Continuous	10.2	18	175	169	397	Martins et al. (2012)
<i>Copaifera langsdorffii</i>	Bees	Continuous	5.6	64	74	39	297	Tarazi et al. (2013)
<i>Copaifera langsdorffii</i>	Bees	Fragmented	23.3	5	94	86	229	Sebbenn et al. (2011)
<i>Copaifera langsdorffii</i>	Bees	Fragmented	23.3	75	63	53	1,420	Manoel et al. (2012)
<i>Cariocaria brasiliensis</i>	Bat	Continuous	12.2	6	132	145	496	Collevatti et al. (2010)
<i>Dicorynia guianensis</i>	Bees	Continuous	3.93	29	142	—	350	Latouche-Hallé et al. (2004)
<i>Diplorenatus</i>	Bees	Continuous	3.94	9	222	—	241	Kenta et al. (2004)
<i>Diplorenatus</i>	Bees	Continuous	3.94	13	192	—	201	Kenta et al. (2004)
<i>Diplorenatus</i>	Bees	Continuous	0.58	20	240	—	306	Hanson et al. (2008)
<i>Diplorenatus</i>	Bees	Fragment	0.21	39	343	—	1,000	Hanson et al. (2008)
<i>Diplorenatus</i>	Bees	Pasture	0.22	34	557	—	2,465	Hanson et al. (2008)
<i>Dysoxylum malabaricum</i>	Insect	Continuous HD	1.72	8	1,205	106	23,600	Ismail et al. (2012)
<i>Dysoxylum malabaricum</i>	Insect	Continuous LD	0.075	—	600	56	—	Ismail et al. (2012)
<i>Dysoxylum malabaricum</i>	Insect	Isolated trees	0.026	—	6,525	5,316	—	Ismail et al. (2012)
<i>Entandrophragma cylindratum</i>	Insect	Unlogged	1.53	70	338	260	1,027	Lourmas et al. (2007)

<i>Entandrophragma cylindrum</i>	Insect	Middle logged	1.13	74	266	156-	944	Lourmas et al. (2007)
<i>Entandrophragma cylindrum</i>	Insect	Severely logged	0.28	66	385	268	2,095	Lourmas et al. (2007)
<i>Gomortega keule</i>	Insect	Large sites	0.005	57	709	—	2,510	Lander et al. (2010)
<i>Gomortega keule</i>	Insect	Small sites	0.005	—	709	—	2,833	Lander et al. (2010)
<i>Gomortega keule</i>	Insect	Single tree	0.005	—	709	—	3,133	Lander et al. (2010)
<i>Guaiacum sanctum</i>	Bees	Continuous	0.70	19	1,276	—	3,500	Fuchs and Hamrick (2011)
<i>Hymenaea courbaril</i>	Bats	Continuous	0.238	55	827	—	1,943	Lacerda et al. (2008)
<i>Hymenaea courbaril</i>	Bats	Continuous	0.101	38	952	869	2,204	Carneiro et al. (2011)
<i>Hymenaea stigonocarpa</i>	Bats	Fragmented	0.0094	31	860	838	7,362	Moraes and Sebbenn (2011)
<i>Hymenaea stigonocarpa</i>	Bats	Isolated trees	0.0094	35	5,229	3,460	3,439	Moraes and Sebbenn (2011)
<i>Kandelia candel</i>	Small insects	Continuous	3,749	31	15.2	—	74	Geng et al. (2008)
<i>Myracrodruon urundeuva</i>	Bees	Fragmented – juveniles	1.08	3	138	64	863	Gaino et al. (2010)
<i>Myracrodruon urundeuva</i>	Bees	Fragmented – seeds	1.08	2	252	192	890	Gaino et al. (2010)
<i>Neobalanocarpus heimii</i>	Bees	Continuous – seedlings	0.83	7	188	—	664	Konuma et al. (2000)
<i>Neobalanocarpus heimii</i>	Bees	Continuous – sampling	0.83	22	195	—	—	Konuma et al. (2000)
<i>Simarouba amara</i>	Bees	Continuous	1.59	42	334	—	1,063	Hardesty et al. (2006)
<i>Swietenia humilis</i>	Bees	Fragment – Las Tablas	1.43	64	—	—	300	White et al. (2002)
<i>Swietenia humilis</i>	Bees	Fragment – Botus	<1	53	—	—	3,100	White et al. (2002)
<i>Swietenia humilis</i>	Bees	Fragment – Joice	<1	62	—	—	1,700	White et al. (2002)
<i>Swietenia humilis</i>	Bees	Fragment – Tablas plains	<1	42	—	—	1,600	White et al. (2002)
<i>Swietenia humilis</i>	Bees	Isolated tree 501	<0.001	100	—	—	>4,500	White et al. (2002)
<i>Swietenia macrophylla</i>	Bees	Continuous – logged	0.9	37	128	117	316	Sebbenn et al. (2012)
<i>Swietenia macrophylla</i>	Bees	Continuous – logged	0.4	0	75	78	128	Sebbenn et al. (2012)
<i>Swietenia macrophylla</i>	Bees	Continuous – logged	0.4	50	176	189	244	Sebbenn et al. (2012)

(continued)

Table 1 (continued)

Tree species	Pollinator	Type of forest	Density (trees/ha)	External pollen flow (%)	Arithmetic mean (m)	Median (m)	Maximum (m)	References
<i>Swietenia macrophylla</i>	Bees	Continuous – logged	0.3	41	255	183	576	Sebbenn et al. (2012)
<i>Sympetrum globulifera</i>	Birds	Continuous – 2002	0.332	0.3	907	–	2,832	Carneiro et al. (2009)
<i>Sympetrum globulifera</i>	Birds	Continuous – 2003	0.332	0.4	963	–	2,658	Carneiro et al. (2009)
<i>Tabebuia aurea</i>	Bees	Continuous	6.5	–	308	–	1,651	Braga and Collevatti (2011)
<i>Tabebuia aurea</i>	Bees	Continuous	6.5	–	396	–	2,484	Braga and Collevatti (2011)
<i>Theobroma cacao</i>	Insect	Continuous	279	61	28	28	67	Silva et al. (2011)

Spearmann rank correlation between tree density and average pollen distance = -0.705 ($P < 0.05$)

Tropical tree populations have high rates of pollen flow among populations. From the studies listed in Table 1 and realized in (a) continuous populations, (b) fragmented populations, as well as (c) with isolated trees in pastures, 72 %, 70 %, and 100 %, respectively, had more than 10 % of pollen immigration. Thus, animal pollination is efficient to promote gene exchange among populations even in fragmented population and for isolated trees in pastures. The average pollen dispersal distance ranged from 12 m to 6,525 m, with the highest value observed of 23.6 km for *Dysoxylum malabaricum*. The pollen dispersal of many tropical trees is characterized by a high proportion of close neighbor pollinations (Bittencourt and Sebbenn 2007; Lacerda et al. 2008; Sebbenn et al. 2011; Lander et al. 2010). This pattern is visible when the median pollen dispersal distance is smaller than the arithmetic mean distances (Table 1). In 19 of 23 studies reporting both arithmetic mean and median pollen dispersal distance, the median was lower than the arithmetic mean. This pattern can be explained by the behavior of the pollinators and flowering density. Some bee species are known to forage preferentially between near neighbors (Degen and Roubik 2004; Dick et al. 2008), and this as a consequence increases the frequency of short-distance pollen dispersal.

Seed dispersal is influenced by the size of the seeds and by the type of vectors. Seed dispersal often occurs in different steps. After a primary random or clumped distribution, a second dispersal further affects seed establishment. Where seeds are deposited depends on the mode of seed dispersal. The literature on the natural history of seed dispersal suggests the following dispersal modes:

Wind: Wind dispersal is present in some canopy tree species (e.g., *Jacaranda copaia*, *Swietenia macrophylla*, *Tachigali* sp., *Schizolobium* sp.) and in many canopy lianas. A variety of studies have modeled the physics of dispersal by wind, and these show a declining density function away from the source tree, often with a directional component from persistent wind patterns (Augspurger 1986; Augspurger and Franson 1987; Matlack 1987; Anderson 1991).

Gravity: In some species, the large, heavy seeds simply fall from the tree to the ground below. These species may represent trees in which the original secondary dispersal agent was a member of the Pleistocene megafauna (principally, giant ground sloths) which are now extinct (Janzen and Martin 1981). Current secondary dispersal agents are probably mostly caviomorph rodents (agoutis, acouchis, pacas). The fruits are too large and woody to be effectively carried away by arboreal or volant vertebrates, and they cannot be opened in the crown to allow dispersal of single seeds. Thus, the primary dispersal mode is by gravity, coupled with additional seed movements affected by terrestrial vertebrates.

Animal Dispersal: A large fraction (perhaps 75 %) of tropical trees have fruits adapted for animal dispersal (Howe and Smallwood 1982).

Birds: There seem to be two principal disperser groups. Large birds consume large fruits and fly to perches or nest sites nearby, where they either regurgitate or defecate seeds below the perch tree. Most seeds are thus deposited in aggregated patterns either below the source tree or below the perch site. Small birds consume smaller fruits, usually passing the seeds within 20 min to 1 h. The defecations are likely to be spread more evenly through their movement ranges, since they probably defecate continuously as they continue to forage and move about.

Bats: Because bats do not perch to eat fruits (as do birds), they must carry the fruit to a feeding roost, where they consume it (or portions of it). If the seeds they eat are small (*Bagassa guianensis*), they are probably defecated either in the roost or in the flight patterns to and from the feeding tree. If the seeds are big, their fruit layer (*Dipteryx odorata*, *Sympomia globulifera*) is consumed and the seeds are dropped below the feeding roost or as the bats fly to and from the source and the roost.

Primates: Primate species vary in the fraction of their diet which is fruit, the foraging behavior in visiting the fruiting trees, and the behavior by which seeds are dropped (or defecated) after foraging. The principal Neotropical primate groups for which foraging has been well studied are *Cebus*,

Ateles, *Alouatta*, and *Saguinus*. While they waste a great deal of fruit by dropping it below the tree (Howe 1980), they can also be effective dispersers (Garber 1986; Estrada and Coates-Estrada 1986).

Ground-Dwelling Rodents: The important dispersers are the caviomorph rodents (agoutis, acouchis, and pacas), which consume some fruits or seeds and scatter hoard other seeds for later consumption. Caching produces small clumps of seeds, more than one of which may germinate. However, over the long term, only one seed from such a cache has a chance of recruiting into the adult population (Howe 1989).

The impact of seed dispersal on the genetic composition of tree populations is determined by the survival of the seed up to the reproductive age. The establishment of seeds is in many cases density- and distance-dependent and controlled by predation and the environment (Condit et al. 2000; Muller-Landau et al. 2008). Ecological studies have shown that post-dispersal mechanisms promote the survival and establishment of long-distance seed dispersal (Nathan and Casagrandi 2004; Tarazi et al. 2013). Nevertheless most studies using genetic markers observed spatially restricted seed dispersal (Vekemans and Hardy 2004; Hardy et al. 2006; Dick et al. 2008; Bittencourt and Sebbenn 2007; Gaino et al. 2010; Sebbenn et al. 2011; Sant'Anna et al. 2013). Studies using maternity analysis in tropical tree species detected average seed dispersal distance ranking from 40 m to 175 m (Table 2).

There are basically two main approaches to study gene flow using molecular markers: (a) an analysis of the realized (historic) gene flow and (b) estimations of the contemporary gene flow (Oddou-Muratorio and Klein 2008). The first approach is based on the measured genetic fixation and differentiation (F_{st}) among and within populations (Hamrick and Nason 2000). The second method uses parentage analysis (maternity and paternity) of open-pollinated seeds and established seedlings, juveniles, or young trees (Aldrich and Hamrick 1998; Oddou-Muratorio and Klein 2008; Bittencourt and Sebbenn 2007; Gaino et al. 2010; Sebbenn et al. 2011; Sant'Anna et al. 2013). Parentage analysis is a direct way to measure the distribution pattern of pollen and seeds in tree species (Dow

Table 2 Results on seed dispersal: arithmetic mean, median, and maximum distance of seed dispersal for some tropical tree species

Tree species	Vector of dispersal	Type of forest	External seed flow (%)	Mean (m)	Median (m)	Maximum (m)	Authors
<i>Araucaria angustifolia</i>	Gravity	Fragmented	0	92	–	291	Bittencourt and Sebbenn (2007)
<i>Araucaria angustifolia</i>	Gravity	Continuous	5	131	133	318	Sant'Anna et al. (2013)
<i>Carapa guianensis</i>	Animal	Continuous	36.7	175	149	397	Martins et al. (2012)
<i>Carapa guianensis</i>	Animal	Continuous	25.4	114	106	334	Martins et al. (2012)
<i>Copaifera langsdorffii</i>	Gravity	Fragmented 1.2 km	0	61	52	170	Sebbenn et al. (2011)
<i>Copaifera langsdorffii</i>	Gravity	Continuous	15	135	–	–	Tarazi et al. (2013)
<i>Jacaranda copaia</i>	Wind	Continuous – 2000	16	40	21	422	Jones and Hubbell (2006)
<i>Jacaranda copaia</i>	Wind	Continuous – 2002	16	59	27	710	Jones and Hubbell (2006)
<i>Myracrodruon urundeuva</i>	Wind	Fragmented	0	124	46	887	Gaino et al. (2010)

and Ashley 1996; White et al. 2002; Burczyk et al. 2004; Kamm et al 2009; Ashley 2010; Sebbenn et al. 2011; Leonarduzzi et al. 2012). Parentage analyses permit the genealogical reconstruction of the relatedness between individuals within and among populations (Burczyk et al. 2004; Ashley 2010; Leonarduzzi et al. 2012). The great advantage of this approach is that no a priori assumptions on the dispersal models are required. But for the parentage analysis, it is necessary to sample all reproductive trees in a specific area and to work with highly variable gene. Because of the high number of alleles, nuclear microsatellites (nSSRs) are the most suitable gene markers for this approach (Ashley 2010). Such studies also permit to estimate and compare the male fertilities of individual trees and to analyze correlations among fertilities and different traits such as the size of the trees. In trees, male mating success has been reported to increase with proximity and tree size (Burczyk et al. 1996; Klein et al. 2008). Significant associations have been found between the diameter of the trees and male fertility: *Araucaria angustifolia* (Bittencourt and Sebbenn 2007, 2008), *Hymenaea courbaril* (Lacerda et al. 2008; Carneiro et al. 2011), *Hymenaea stigonocarpa* (Moraes and Sebbenn 2011), and *Copaifera langsdorffii* (Sebbenn et al. 2011; Tarazi et al. 2013).

Spatial Genetic Structure

Many tropical tree species disperse seeds close to the mother tree (Asuka et al. 2005; Bittencourt and Sebbenn 2007; Geng et al. 2008; Nakanishi et al. 2008). Seedlings tend to grow around their mother tree, representing a mixture of different full- and half-sib families (Asuka et al. 2005; Bittencourt and Sebbenn 2007; Geng et al. 2008; Nakanishi et al. 2008; Sebbenn et al. 2011, see also Table 4). We speak about an intrapopulation spatial genetic structure (SGS) in case that the genotypes are not randomly distributed, but trees that are closer to each other are genetically more similar. The SGS can be created by a limited pollen and seed dispersal (Loiselle et al. 1995; Vekemans and Hardy 2004; Cavers et al. 2005), by vegetative propagation (Silva et al. 2011), and also by microhabitat selection. The intensity of the SGS and the spatial extension need to be considered in all sampling activities in order to do genetic studies on diversity, to collect material for gene conservation, or to harvest seeds to produce reproductive material for plantations and breeding programs. The overall objective is to avoid sampling of individuals that are relatives with a narrow genetic basis.

The spatial genetic structure can be studied using codominant gene markers (allozymes, microsatellite, RFLP, and SNPs) and dominant gene markers (AFLP and ISSR). For each collected tree the spatial position gets registered, and based on the genotypes and haplotypes screened at the gene markers, different statistics are computed to quantify the spatial genetic structure. Different software programs are available for that: SPAGeDi (Hardy and Vekemans 2002), SGS (Degen et al. 2000), and GenAIEx (Peakall and Smouse 2006). The statistics on the spatial genetic structure compute for pairs of individuals belonging to different spatial distance classes by either spatial autocorrelations (e.g., Moran's I), measures of genetic relatedness (Queller and Goodnight 1989; Lynch and Ritland 1999), coefficient of coancestry (Loiselle et al. 1995; Ritland 1996), or genetic distances (Gregorius 1978).

The ability to detect the SGS in a population of tree species depends strongly on the type of gene marker used and on the sampling strategy (Cavers et al. 2005). For studies using AFLP and microsatellite marker, at least 100 individuals for 10 microsatellite loci and 150 individuals for 100 AFLP loci should be used. The necessary plot size is a function of population density. In high-density populations smaller plots will be necessary compared to low-density populations (Table 3). For example, for 10 microsatellite loci and aiming to sample 100 trees in a species with 100 individuals per hectare, the minimum limit of the plot will be 1 ha. In contrast, for a low population

Table 3 Observed extension of spatial genetic structure for some tropical tree species

Species	Vector of seed dispersal	Studied stage	Density (trees/ha)	Extension of SGS (m)	References
<i>Araucaria angustifolia</i>	Gravity	Adults	42.2	50	Bittencourt and Sebbenn (2007)
<i>Araucaria angustifolia</i>	Gravity	Seedlings	12.2	50	Bittencourt and Sebbenn (2007)
<i>Araucaria angustifolia</i>	Gravity	Juveniles	17.0	50	Bittencourt and Sebbenn (2007)
<i>Araucaria angustifolia</i>	Gravity	Adults	7.7	75	Bittencourt and Sebbenn (2008)
<i>Araucaria angustifolia</i>	Gravity	Adults	33.4	70	Mantovani et al. (2006)
<i>Araucaria angustifolia</i>	Gravity	Adults	40.3	20	Sant'Anna et al. (2013)
<i>Araucaria angustifolia</i>	Gravity	Juveniles	31.0	20	Sant'Anna et al. (2013)
<i>Araucaria angustifolia</i>	Gravity	Adults	33.4	25	Patreze and Tsai (2010)
<i>Araucaria angustifolia</i>	Gravity	Adults	>20	42–101	Stefenon et al. (2008)
<i>Aucoumea klaineana</i>	Wind	Adults	187.5	<1,000	Born et al. (2008)
<i>Bagassa guianensis</i>	Animal	Adults + juveniles	0.14	300	Silva et al. (2008)
<i>Carapa guianensis</i>	Animal	Seedlings	>10	60	Cloutier et al. (2007a)
<i>Copaifera langsdorffii</i>	Animal	Adults	23.3	50	Sebbenn et al. (2011)
<i>Copaifera langsdorffii</i>	Animal	Seedlings	26.8	20	Sebbenn et al. (2011)
<i>Copaifera langsdorffii</i>	Animal	Juveniles	103.5	40	Tarazi et al. (2013)
<i>Dicorynia guianensis</i>	Gravity	Adults – seedlings	4.3	110	Latouche-Halle' et al. (2003)
<i>Dipteryx panamensis</i>	Animal	Adults	0.21	400	Hanson et al. (2008)
<i>Hymenaea courbaril</i>	Gravity	Adults	0.238	450	Lacerda et al. (2008)
<i>Hymenaea courbaril</i>	Gravity	Juveniles	0.15	300	Lacerda et al. (2008)
<i>Jacaranda copaia</i>	Wind	Adults	6.4	200	Jones and Hubbell (2006)
<i>Manilkara huberi</i>	–	Adults	1.47	399	Azevedo et al. (2007)
<i>Myracrodroon urundeuva</i>	Wind	Adults	1.08	42	Gaino et al. (2010)
<i>Myracrodroon urundeuva</i>	Wind	Juveniles	1.0	32	Gaino et al. (2010)
<i>Simarouba amara</i>	Animal	Seedlings	12.5	140	Hardesty et al. (2005)
<i>Simarouba amara</i>	Animal	Juveniles	12.5	40	Hardesty et al. (2005)

(continued)

Table 3 (continued)

Species	Vector of seed dispersal	Studied stage	Density (trees/ha)	Extension of SGS (m)	References
<i>Swietenia macrophylla</i>	Gravity	Adults	0.3–0.9	150	Sebbenn et al. (2012)
<i>Symponia globulifera</i>	Animal	Adults	10.9	150	Degen et al. (2004)
<i>Symponia globulifera</i>	Animal	Adults + juveniles	0.332	100	Carneiro et al. (2007)
<i>Tabebuia aurea</i>	Wind	Adults	6.5	300	Braga and Collevatti (2011)
<i>Theobroma cacao</i>	Animals	Adults	279	15	Silva et al. (2011)

Table 4 Recommendation for minimum and maximum size of square plots for studies on the spatial genetic structure in tropical tree species with different tree density using microsatellite gene marker

Minimum limit = 100 trees			Maximum limit = 200 trees		
Density (trees/ha)	Plot size (ha)	Square plot	Density (trees/ha)	Plot size (ha)	Square plot
100	1	100 × 100 m	100	2	141 × 141 m
50	2	141 × 141 m	50	4	200 × 200 m
25	4	200 × 200 m	25	8	283 × 283 m
10	10	316 × 316 m	10	20	447 × 447 m
5	20	447 × 447 m	5	40	632 × 632 m
1	100	1,000 × 1,000 m	1	200	1,414 × 1,414 m
0.5	200	1,414 × 1,414 m	0.5	400	2,000 × 2,000 m
0.1	1,000	3,162 × 3,161 m	0.1	2,000	4,472 × 4,472 m

density, as it is the case for many tropical tree species, with 1 or 0.1 tree/ha we are talking about needed plots with a size of 100–1,000 ha.

Many studies have already studied the SGS of tropical tree populations. We summarized the results of some of these studies (Table 4). Generally tree species with wind as a vector of seed dispersal and low population density have larger and more extended SGS compared to other tree species. We found a significant negative Spearman rank correlation among density and average distance of SGS ($r = -0.563$, $P < 0.05$). Tree species with more than 5 trees/ha had in general SGS significantly lower than 100 m.

Mating System in Tropical Tree Species

Mating system defines the pattern in which the gametes of individuals of a population are merged during the reproduction. The mating system is primarily depending on the sexual system. Here we distinguish among (a) unisexual = dioecious species and (b) bisexual species. For dioecious species we have individuals with either male or female sex organs. The bisexual tree species are subdivided into monoecious species with different male and female flowers on the same tree and hermaphroditic species with flowers that produce both male (pollen) and female gametes (ovules). Besides these “pure” bisexual types, we have different combinations of flower types (androdioecism and gynodioecism).

For the dioecious tree species, only crossings among different individuals (outcrossing) are possible, whereas bisexual plant can have different levels of outcrossing and selfing.

The mating system of a tree population has a strong impact on the genetic relatedness of the offspring and on the genetic diversity of the seeds. Thus this information is essential for the design of any sampling strategies either for seeds harvest for future plantations, for gene conservation, or for breeding programs (Sebbenn 2002). Species with high levels of outcrossing are expected to have a lower genetic differentiation among populations compared to species that have high levels of self-fertilization. Outcrossing leads to a stronger recombination of genetic information and thus higher level of genetic variation in each generation (Hamrick 1983). The recombination of gametes by outcrossing reduces the level of homozygosity and thus the risk that recessive deleterious alleles get homozygote.

Following Goodwillie et al. (2005), the mating system in plants can be classified according to the outcrossing rate (t) as (a) autogamy ($t < 20\%$), (b) outcrossed ($t > 80\%$), or (c) as a mixed mating system ($20\% > t < 80\%$). For tropical tree species a wide range of different mating systems have been observed, including different levels of outcrossing, selfing, and mating among relatives. Also asexual reproduction can occur, e.g., by apomixes as detected in *Jatropha curcas* (Bressan et al. 2013). Initially, it was assumed that tropical tree species were self-compatible and produced seeds primarily by selfing (Federov 1966). The theory was that the low densities of individual trees per species would then lead to low levels of genetic diversity and high inbreeding. Later studies of Bawa (1974), Ashton (1976), and Bawa et al. (1985) using controlled crossings and other studies using gene markers (Table 1) showed that this theory was wrong. Apparently there is a predominance for outcrossing in tropical tree species, and there is a strong selection against inbreds stabilizing high levels of genetic diversity. Bawa (1974) analyzed the breeding system of tree species in the tropical semi-deciduous forest of Costa Rica. With controlled pollinations and direct

Table 5 Results of mating system parameters in some tropical tree species

Tree species	Type of forest	Density (trees/ha)	t_m	$t_m - t_s$	r_p	Θ	N_e	m	References
<i>Araucaria angustifolia</i>	Continuous	201	1.00	0.05	0.21	0.15	3.31	45	Sousa et al. (2005)
<i>Araucaria angustifolia</i>	Exploited	166	1.00	0.02	0.40	0.17	2.86	52	Sousa et al. (2005)
<i>Araucaria angustifolia</i>	Continuous	>57	1.00	0.04	0.40	0.17	2.87	52	Sousa et al. (2005)
<i>Araucaria angustifolia</i>	Continuous	>57	1.00	0.06	0.23	0.15	3.24	46	Sousa et al. (2005)
<i>Araucaria angustifolia</i>	Continuous	7.7	1.00	0.11	0.20	0.17	2.87	46	Bittencourt and Sebbenn (2008)
<i>Araucaria angustifolia</i>	Planted	>200	1.00	0.02	0.24	0.15	3.10	48	Ferreira et al. (2012)
<i>Araucaria angustifolia</i>	Planted	>200	1.00	0.02	0.03	0.13	3.80	39	Ferreira et al. (2012)
<i>Araucaria angustifolia</i>	Continuous	>50	1.00	0.06	0.44	0.18	2.70	56	Ferreira et al. (2012)
<i>Bagassa guianensis</i>	Continuous	0.14	1.00	0.01	0.14	0.15	3.40	29	Silva et al. (2008)
<i>Carapa guianensis</i>	Continuous	2.5	0.94	0.01	0.05	0.20	2.48	40	Cloutier et al. (2007b)
<i>Carapa guianensis</i>	Logged	2.0	0.93	0.03	0.05	0.20	2.48	40	Cloutier et al. (2007b)
<i>Carapa guianensis</i>	Continuous	25.7	0.86	0.13	0.08	0.18	2.70	56	Campos et al. (2013)

(continued)

Table 5 (continued)

Tree species	Type of forest	Density (trees/ha)	t_m	$t_m - t_s$	r_p	Θ	N_e	m	References
<i>Cariniana legalis</i>	Fragmented	0.90	0.99	0.10	0.32	0.21	2.39	63	Sebbenn et al. (2000)
<i>Cariniana legalis</i>	Fragmented	<1	0.99	0.06	0.29	0.21	2.42	62	Sebbenn et al. (2000)
<i>Cariniana legalis</i>	Fragmented	<1	0.90	0.00	0.21	0.21	2.36	64	Sebbenn et al. (2000)
<i>Copaifera langsdorffii</i>	Fragmented	5.2	0.86	0.08	0.06	0.17	2.65	57	Tarazi et al. (2013)
<i>Copaifera langsdorffii</i>	Board	<1	0.76	0.08	0.06	0.20	2.30	65	Tarazi et al. (2013)
<i>Eucalyptus rameliana</i>	Continuous	>10	0.89	–	0.09	0.16	3.07	49	Sampson (1998)
<i>Hymenaea courbaril</i>	Continuous	0.24	1.00	0.10	0.27	0.21	2.39	42	Lacerda et al. (2008)
<i>Guaiacum sanctum</i>	Continuous	0.70	0.95	0.10	0.51	0.19	2.56	59	Fuchs and Hamrick (2011)
<i>Guaiacum sanctum</i>	Fragmented	<0.70	0.72	0.90	0.78	0.25	1.96	77	Fuchs and Hamrick (2011)
<i>Hymenaea courbaril</i>	Logged	0.10	0.96	0.07	0.14	0.17	2.66	56	Carneiro et al. (2011)
<i>Hymenaea courbaril</i>	Fragmented	<0.1	0.98	0.29	0.18	0.19	2.63	57	Feres et al. (2009)
<i>Jatropha curcas</i>	Planted	900	0.68	0.35	0.99	0.35	1.43	105	Bressan et al. (2013)
<i>Manilkara huberi</i>	Continuous	1.47	1.00	0.28	0.20	0.20	2.50	40	Azevedo et al. (2007)
<i>Myracrodroon urundeuva</i>	Fragmented	1.08	1.00	0.01	0.10	0.13	2.85	53	Gaino et al. (2010)
<i>Pachira quinata</i>	Continuous	>1	0.91	–	0.47	0.20	2.54	59	Fuchs et al. (2003)
<i>Pachira quinata</i>	Isolated trees	<0.5	0.78	–	0.74	0.24	2.06	73	Fuchs et al. (2003)
<i>Senna multijuga</i>	Continuous	<1	0.54	0.05	0.25	0.28	1.81	83	Ribeiro and Lovato (2004)
<i>Senna multijuga</i>	Pastures	>10	0.84	0.11	0.31	0.20	2.56	59	Ribeiro and Lovato (2004)
<i>Sextonia rubra</i>	Logged	0.95	0.99	–	0.10	0.16	3.03	50	Veron et al. (2005)
<i>Sympomia globulifera</i>	Logged	10.9	0.92	0.16	0.47	0.18	2.73	37	Degen et al. (2004)
<i>Sympomia globulifera</i>	Continuous	0.83	1.00	0.10	0.41	0.19	2.60	58	Carneiro et al. (2007)
<i>Sympomia globulifera</i>	Continuous	0.83	1.00	0.10	0.39	0.19	2.65	57	Carneiro et al. (2007)
<i>Swietenia macrophylla</i>	Logged	0.51	0.90	0.12	0.40	0.20	2.27	44	Lemes et al. (2007)
<i>Tabebuia rosea alba</i>	Fragmented	<1	0.84	0.37	0.12	0.32	1.49	100	Feres et al. (2012)
<i>Tabebuia rosea alba</i>	Planted	10	0.96	0.40	0.10	0.32	1.47	102	Feres et al. (2012)
<i>Theobroma grandiflorum</i>	Continuous	<1	1.00	0.05	0.93	0.24	2.07	48	Alves et al. (2003)
<i>Theobroma cacao</i>	Continuous	279	0.96	0.03	0.19	0.15	2.93	51	Silva et al. (2011)

observations of the floral biology, he found that from 130 tree species, 68 % were hermaphrodites, 10 % were monoecious, and 22 % were dioecious species. Bawa et al. (1985) observed that 86 % of the hermaphrodite and monoecious species showed self-incompatibility mechanism, which successfully eliminates or avoids selfing in tropical tree species. These mechanisms explain the high rates of outcrossing ($t_m = 0.88$) detected in many tropical tree species (Table 5).

The mating system varies among different tree species but also among different populations or individuals of the same tree species (Table 5). Examples for variation among different individuals of the same population are reported for *Platypodium elegans* (Hufford and Hamrick 2003) and for

Magnolia stellata (Tamaki et al. 2009). Temporal variation of outcrossing rates of the same species and population at different reproductive events have also been observed for *Platypodium elegans* (Hufford and Hamrick 2003), and even variations of outcrossing rates among and within fruits of the same individuals are published (e.g., *Acacia melanoxylon*, Muona et al. 1991; *Eucalyptus rameliana*, Sampson 1998; *Magnolia stellata*, Tamaki et al. 2009). In animal-pollinated tropical tree species, mating systems have been shown to be affected by factors such as the density of reproductive trees (Murawski and Hamrick 1991) and the pollinator behavior (Hirao et al. 2006), by forest fragmentation and the level of spatial isolation of trees (Cascante et al. 2002; Fuchs et al. 2003; Lowe et al. 2005; Aguilar et al. 2008; Feres et al. 2012), and by logging activities (Doligez and Joly 1997; Lacerda et al. 2008; Carneiro et al. 2011). Both the temporal isolation (no overlapping in flowering period of trees) and spatial isolation of flowering (low density of flowering trees) are correlated to high rates of self-pollination for some tree species (Murawski and Hamrick 1991; Dick et al. 2003; Naito et al. 2008; Moraes and Sebbenn 2011). Forest fragmentation and selective logging reduce the density of reproductive individuals and may affect the behavior of pollinators. For some species this had an impact on the outcrossing rate and the number of effective pollen donors leading to higher inbreeding and an increased in the coancestry within progenies (Table 5).

Forest fragmentation modifies the movement of pollen and seeds within tree populations and has a negative impact on ecological and evolutionary processes (Cuartas-Hernandez et al. 2010). These disturbances may lead to lower rates of outcrossing, lower genetic diversity in the seeds, and increased mating among relatives and selfing (White et al. 2002; Burczyk et al. 2004; Sebbenn et al. 2011; Breed et al. 2012). Trees that have usually a high proportion of outcrosses can accumulate deleterious recessive alleles (Ward et al. 2005; Petit and Hampe 2006). This is a genetic load which will be effective when the inbreeding increases (Lowe et al. 2005; Breed et al. 2012). High rates of selfing have been found in extremely low-density tree populations, small forest fragments, and in isolated trees in pastures (Dick et al. 2003; Lander et al. 2010; Moraes and Sebbenn 2011; Manoel et al. 2012; Tarazi et al. 2013). Most of these high levels of inbreeding are explained with changes in the foraging behavior of the pollinators (Degen and Roubik 2004; Harder and Barrett 1995; Ghazoul 2005):

t_m is the multilocus outcrossing rate. $t_m - t_s$ is an estimator for the mating among relatives. $r_{p(m)}$ is the correlation of paternity = an estimator for the proportion of full-sibs within a progeny; Θ is the coefficient of coancestry within progeny. N_e is the variance effective population size within progeny. m is the number of seed trees to be collected in order to reach the target effective population size of 150 for gene conservation programs (Lacerda et al. 2008). Spearman rank correlation among tree density and multilocus outcrossing rate = -0.317 ($P > 0.05$).

How to Measure Parameters of the Mating System?

A common approach is the application of the so-called “mixed mating” model of Ritland and Jain (1981). For this approach single tree progenies are collected in natural forests or seed orchards. The seeds are then genotyped with codominant gene markers such as microsatellites or allozymes. The data set gets analyzed with the MLTR software (Ritland 2002). Different parameters are computed including the multilocus outcrossing rate (t_m), selfing rates, and the proportion of mating among relatives ($t_m - t_s$). The program can also compute the effective number of pollen donors ($N_{ep} = 1/r_{p(m)}$, Ritland 1989) and average coancestry coefficient (Θ) within progenies. Based on the statistics we can also estimate the number of seed trees (m) to be collected to maintain genetic diversity in gene conservation programs (Sebbenn 2006).

Usually open-pollinated progenies from a limited number of seed trees are collected for a mating system study. The seeds and the seed trees are genotyped with gene markers. According to Ritland

and Jain (1981), 4–5 codominant gene loci (allozymes, microsatellite, or SNPs) with intermediate allele frequencies (~0.5) are enough. If the frequencies of alleles at the loci are high (>0.8), higher number of loci should be included in the study. We suggest to collect at least 30 seeds per tree from at least 25 open-pollinated seed trees. If the tree species have fruits with more than 1 seed per fruit, then the 30 seeds per tree should be collected in a balanced way including seeds from different fruits (e.g., 5 or 10 seeds per fruit if possible) (Muona et al. 1991; Sampson 1998; Silva et al. 2011; Feres et al. 2012; Bressan et al. 2013). This design enables a hierarchical analysis of mating parameters. Generally we suggested to collect seeds from at least 25 seed trees per population and to repeat the sampling at different reproductive event (temporal variation) and in different populations (spatial variation). Such a detailed study of the mating system of a species including genetic inventories of seeds from different populations and different reproductive periods is very labor-intensive and costly. If more than two populations are sampled, we suggest according to Ritland (2002) a minimum sample size of 15 seed trees per populations and at least 20 seeds per seed trees.

Tree Breeding

Introduction

A wide variety of different tree species and provenances are used for plantations and reforestation in the tropics. Intensively used for plantations are different *Eucalyptus* species and tropical pines because of their fast growth and good adaptation to different kinds of soil and climates. *Eucalyptus* species of great use are *E. grandis*, *E. urophylla*, *E. dunnii*, *E. camaldulensis*, *E. citriodora*, *E. saligna*, *E. globulus*, *E. tereticornis*, *E. benthamii*, *E. pilularis*, *E. maculata*, *E. cloeziana*, *E. pellita*, *E. viminalis*, *E. deanei*, *E. resinifera*, and the classic hybrid *E. grandis* x *E. urophylla* (*E. urograndis*). Pines species intensively planted are *P. caribaea* var. *bahamensis*, *P. caribaea* var. *caribaea*, *P. caribaea* var. *hondurensis*, *P. oocarpa*, and *P. tecunumanii*. Other tropical of broad use are *Araucaria angustifolia*, *Tectona grandis*, and *Hevea brasiliensis*.

There are basically three steps in a classical domestication or breeding program for tree species: (i) selection of species, (ii) selection of the most productive and adapted provenances of each species, (iii) and progeny tests (Fig. 1). The species are selected according to the main purpose of the plantations (e.g., paper and pulp production, saw timber or energy production) and according to the environments in the region of plantations. The most suitable tree species get identified in frame of tree species tests. These are field trials with different tree species planted in the target region. The second stage is then the selection of the most productive and adapted seed sources (provenances) within a tree species using provenance test. Often species and provenance tests are combined. Usually we expect large differences in growth, resistance, and phenology for tree species with a large natural distribution area covering quite variable environments. The last stage is the selection of the best individuals within the best provenances. These individuals are either directly further tested and planted as clones after vegetative propagation or they are used as parents in controlled crosses. Then the genetic advantage of the selected individuals is estimated by the performance of their progenies.

Selection of the Site Test

The sites for the trials should be representative for the environments of the target regions of future plantations (Eldridge et al. 1993). The determination of the sites can be made based on a classification of soils, climate, and altitude. The most important climatic factors to be considered are the minimum and maximum temperature, precipitation, and the occurrence and timing of dry seasons throughout the year. The alkalinity, salinity, acidity, and nutrient content of the soil have also a great

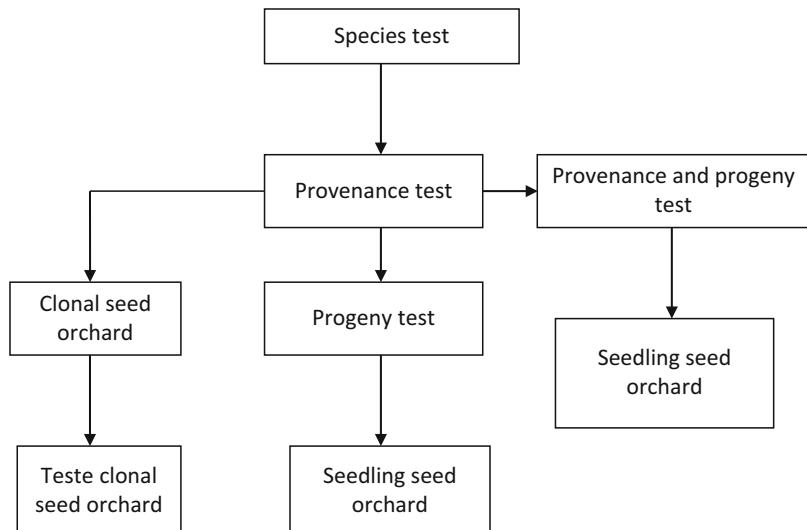


Fig. 1 Different steps and stages of a "classical" tree breeding program

influence on the distribution and adaptation of species. The location of the trial test should be set randomly in each of relevant environment classes. Within a test side the environment should be as homogeneous as possible in order to get a clear result.

Provenance Test

The term "provenances" is synonymous for the seed source of origin (Eldridge et al. 1993; Pedersen et al. 1993). Usually provenance tests include seed sources collected from many parts of the natural range of a species. Important features that may vary among provenances are the growth rate; stem form; resistance to frost, pollution, fungi, pests, and other diseases; the ability to tolerate salinity, alkalinity, and acidity; or the performance in face of excess or lack of moisture in soil (Pedersen et al. 1993).

Provenance tests have a great importance on the selection of appropriate material for plantations and reforestation (Lindgren and Persson 1997; Zobel and Talbert 1984). The difference among populations of a species may be so great that plantings made with a particular source of seeds can result in big successes and seeds originating from other sources of the species may result in complete failures. Therefore, it is essential that foresters know the origin of the seeds used in their plantations (Eldridge et al. 1993). However, in cases of native species, it is recommended as a general rule the use of local provenances until the results of provenance tests are available and indicate nonlocal origins as advantageous. Based on correlations between juvenile and adult stages, decisions on the recommended seed sources should be done when the field trials have been measured at least half of the rotation period (Zobel and Talbert 1984; Eldridge et al. 1993; Pedersen et al. 1993). At this age the ranking of the performance of the different provenances gets stable.

The number of provenances included in the field trial should represent the amount of genetic variation within the target species. Often provenance tests include 10–30 different seed sources. It makes sense to select provenances along environmental gradients including the ecological optimum of the species and its extremes (Pedersen et al. 1993). Often gradients are represented by differences in latitude and altitude. Many provenance trials use the same seed sources as common standard. This might be a seed source that is already of commercial importance on the market.

Each provenance should be genetically representative for the seed sources. The sampling in a population should include at least seeds from 15 different trees and if possible seeds from more than

25 trees. There should be a minimum distance among sampled trees of 100 m for high-density species and more than 300 m for low-density tree species in order to avoid the collection of genetically related material (Pedersen et al. 1993). In practice, the number of parent trees where seeds are collected to represent a provenance will depend on the number of provenances to be sampled. If few provenances (3–4) are evaluated, the number of seed trees for seed collection can be 50. If many provenances are included in the test, then the collection of seeds from 15 to 25 trees may be sufficient. The seed trees for provenance test could be selected according to stem form, diameter at breast height (DBH), height, volume, wood density, resin production, pest, and diseases. Often dominant trees, free of diseases and pests, and inferior trees (plus trees) are included in the samples (Eldridge et al. 1993; Pedersen et al. 1993). You should also collect many seeds from each tree in order to obtain sufficient material for field test. For a given provenance the seeds of different seed sources should be equally represented. Thus a mixture of equal quantities of seeds is required. For example, if seeds are collected from 15 seed trees and there are great differences for the number of harvested seed varying from 2 to 5 kg, then from each tree 2 kg should be mixed.

If you have a series of provenance trials with the same provenances planted in different environments, then the so-called genotype environment interaction can be studied. The ranking of the performance of provenances often changes in different environments. Some provenances are stable. Thus the recommendations on the most suitable provenances could either focus on the best provenance for each environment (in case the environment is supposed to be stable at plantation side) or focus on stable provenances in case of expected high variation of environment. It is noteworthy that the selecting of specific provenances for each environment will lead to higher costs and efforts but has the advantage of optimizing the genetic gains for each environment and maintain the genetic variability.

Provenance test must be established using a specific experimental design in order to estimate the influence of genetic and environmental effects on the phenotypes of the trees (Eldridge et al. 1993). Usually the same design and the same provenances are used in a series of trials at different locations. Within a given side the small scale environmental variation is estimated by repetitions of the same provenances at different locations within side. A simple way is to subdivide the trial in 3–10 blocks and plant every provenance with the same number of individuals in each of the blocks. The accuracy is proportional to the square root of the number of repetitions, but more repetitions result in higher cost for the establishment, maintenance, and measurement of the provenance trial (Pedersen et al. 1993). The number of plants per provenance at a plot (repetition) can vary from 1 plant to more than 50 plants. Generally 5–10 trees per provenance and plot are used to allow a good evaluation of phenotypic variation within provenance. The plots may be of linear, square, or rectangular shape. Linear plots occupy less space but can sometimes be biased by the effects of competition among trees. Therefore, some tests use square plots and measure only the central trees.

Progeny Test

The progeny test aims to evaluate the performance of the known parent by the performance of their descendants (sibs) growing in a controlled environment. Thus, the information obtained in the test serves to affirm which of the known parents present the best gene combination with a positive effect on the target traits (growth, resistance tree form, chemical contents, etc.). The results of the progeny tests can also directly serve to select superior trees from the progenies to establish a seed (seedling seed orchard).

There are basically three types of progeny tests: (i) open-pollinated progeny test, (ii) the polycross progeny test, and (iii) progeny of controlled pollination or full-sib progeny test. The open-pollinated progeny test is conducted from seeds collected from selected seed trees without any control of the

pollination process. The seeds collected from a particular tree may contain different degrees of genetic relation (half-sibs, full-sibs, self-sibs, and self-half-sibs (Squillace 1974)). The open-pollinated progeny test is the most commonly used variant based on plus trees. This is a rapid and low-cost design, because you do not need to make labor-intensive controlled crosses to produce the progenies. This method exploits the general combining ability (GCA). For tests with open-pollinated progenies and thus with seeds of variable level of relatedness, specific statistical models to estimate the genetically determined variation (heritability) are used (Namkoong 1966; Squillace 1974; Miranda et al. 2013). In polycross progeny tests the pollen for the crosses has been collected at different parent trees and get mixed before the controlled crosses (pollination). Thus this approach involves manual pollination and thus is more cost-intensive. This type of test also only explores the general combining ability (GCA) of the mother trees, but the specific role of pollen donors can explicitly be taken into account into the statistical analysis. Finally the full-sib progeny test is a trial using controlled crosses among selected trees, producing full-sib progenies. The costs involved in this approach are larger than the other two methods. However, this approach exploits both the general combining ability (GCA) and specific combining ability (SCA), as both maternal and paternal parents are known. This test is usually used in advanced stages of breeding. Same as for the provenance trials, the progeny tests need to be set up with repetitions (different locations + repetitions within each location). Those parent trees that have been identified in the progenies tests to have most positive genetic effect on the progenies are then selected and used for the following cycles of the breeding program. At different stages superior individuals can be selected and multiplied, e.g., by vegetative propagation for broadscale plantations.

Gene Conservation

In many tropical regions we have a fast deforestation. Remaining forests get more and more fragmented. Selective logging reduces the number of reproductive trees. Natural forests with native tree species are replaced by plantations with exotic tree species. Finally the climate change is expected to have a strong impact on the species composition in the tropics. All these developments endanger the genetic diversity of native tree species. Thus gene conservation is becoming a more and more important measure to maintain the valuable genetic diversity. There are basically two ways to conserve the genetic diversity of tree populations: the in situ and ex situ gene conservation. The in situ approach aims to conserve tree populations with a sufficient large size in the natural distribution area. The in situ gene conservation should always be the first choice because this conservation is not static and allows the population genetic processes like genetic selection to continue. Thus the genetic composition of in situ gene conservation units is still subject to ongoing adaptations. But there are situations where populations are of urgent extinction risk in their natural habitats. Here the ex situ gene conservation is the only alternative. For this seeds or other reproductive material are collected in the remaining parts of the populations and used for the establishment of stands or seed orchards outside the natural distribution range. For the practical realization of the in situ gene conservation, it is very important to know the minimum number of individuals and thus the minimum size of the protected area. For the ex situ gene conservation, the number of individual trees from which reproductive material (seeds or material for vegetative propagation) needs to be collected is essential.

Sample Sizes for Ex Situ Gene Conservation

The minimum number of individuals for the ex situ gene conservation can be estimated based on the level of genetic diversity that should be protected. Due to the sampling effect (genetic drift), especially rare genetic variants (alleles) get lost. There is a long debate on the adaptive relevance of rare alleles. In situations of environmental stress such as high levels of air pollution, studies of tree species in southwestern Europe have suggested that extremely low-frequency alleles (0.001) can have adaptive significance (Krusche and Geburek 1991). Most often alleles with at least 5 % frequency have been defined as the threshold of genetic diversity that should be maintained (Gregorius 1980; Namkoong 1988; Krusche and Geburek 1991; Brown and Hardner 2000). These authors have elaborated formulas that compute minimum numbers of trees under different assumptions. Gregorius (1980) concluded that a sample size of 117 individuals would be enough to maintain a 95 % probability of at least one copy of all alleles with a frequency of at least 0.05. Namkoong (1988) and Krusche and Geburek (1991) estimated that the sampling of 90 individuals from a single population would be sufficient to keep the same allele frequency (5 %) simultaneously at 100 loci. Finally Brown and Hardner (2000) suggested a sample size of 50 individuals to keep alleles with at least 5 % in the populations. It is important that seeds or other reproductive material is collected from individuals that are not relatives. Thus the minimum number of 117 trees for ex situ conservation means to sample seeds from at least 117 unrelated trees.

Effective Population Size (N_e)

For both the in situ and the ex situ gene conservation, the concept of effective population size (N_e) is of high relevance. The effective population size is defined as the number of individuals in an idealized population in which all individuals contribute equally to the set of gametes or have the same variation in allele frequencies or have the same levels of inbreeding (Wright 1931). The definition of N_e in case of variable allele frequencies is defined as the variance effective size. N_e based on the inbreeding is called the inbreeding effective population size (Kimura and Crow 1963). For trees, the variance effective population size has been broadly applied for ex situ gene conservation because it provides an estimation of the number of seed trees and number of seeds per tree that need to be collected. The reference population size is the target N_e and refers to a hypothetical population of infinite size ($N > 10,000$) in Hardy-Weinberg equilibrium (random mating), without effects of selection, mutation, and migration. But this means that in practice, the value N needs to be set higher because we usually have a certain level of inbreeding, overlapping of generations, family structure, variation in phenology, and nonrandom mating. Based on the concept of effective population size, Frankel and Soule (1981) determined the minimum number of individuals required to conserve the adaptive capacity of a population with 50 individuals for short-term conservation (10 generations) and 500 individuals for long-term conservation (100 generations). But these values have been criticized (Nunney and Campbell 1993), because they do not consider other factors that may cause the extinction of a population. For long-term in situ conservation programs, Lynch (1996) proposed that $N_e = 1,000$ individuals would be required to maintain normal adaptive potential of a species.

Thus let us assume that $N_e = 1,000$ is a conservative estimation for an in situ gene conservation program. How many different individuals are required to reach this target value? Studies made in remaining forest fragments of *Araucaria angustifolia* (5.4 ha) and *Myracrodruon urundeuva* (500 ha) in Brazil measured the effective population sizes as values between 121 and 335 (Bittencourt and Sebbenn 2007; Gaino et al. 2010). Thus, these remaining forest fragments are supposed to be too small for long-term gene conservation. For ex situ gene conservation programs, an effective population size (N_e) of 150 is recommended Lacerda et al. (2008). In order

to reach this value Sebbenn (2006) and Moraes and Sebbenn (2011) estimated that seeds need to be collected from 29 to 105 seed trees.

DNA Controls to Reduce Illegal Logging

Illegal logging and trade with illegal timber and wood products are the cause for many economic and ecological problems both in the producer and in the consumer countries. Illegal logging is believed to be one of the chief causes of worldwide deforestation, and trade with illegal timber and wood products creates market disadvantages for products from sustainable forestry. Moreover, fallow land produced by illegal logging contributes to climate change by releasing greenhouse-relevant gases. The OECD assesses global damages through illegal timber at approximately €15 billion/year. According to estimates, approximately 50 % of timber exports from the tropical Amazon Basin, Central Africa, and Southeast Asia originate from illegal logging. Now there are several laws and regulations that aim to reduce illegal logging and the trade with illegally harvested timber. The most important are the EU Timber Regulation that got in force in March 2013, the Lacey Act in the USA, and the Australian Illegal Logging Prohibition Act. All three laws ask for correct claims on the scientific species name and the country of origin. Moreover an increasing number of tree species get protected by listing them at the “Convention on International Trade in Endangered Species of Wild Fauna and Flora” (CITES).

In the past only wood anatomical methods have been made available to identify species identity for many of the traded tree species. But since a few years, molecular markers and DNA sequencing are more and more applied to check claims on tree species and origin (Lowe and Cross 2011).

Species Identification

DNA bar coding is a genetic approach to distinguish between different species. Here differences of the nucleotide sequence at specific target-DNA regions are used for identification. These target-DNA regions show genetic differences among different species but not or only to a small degree within different individuals of a species (Taberlet et al. 2007; Kress et al. 2005; Hebert et al. 2003; Hebert and Gregory 2005). Other characteristics required from these target-DNA regions are that they are present in most taxa and that they are easy to sequence. For species that perform photosynthesis, the so-called ITS region (Internal Transcribed Spacer) of the nucleus has been successfully used for taxonomic purposes during the last 10 years (Syring et al. 2007; Mort et al. 2007; Kencier et al. 2005). The genome of the chloroplast of plants is highly conserved in terms of size (120–170 kb), structure, and linear order of genes. Taxonomists have used sequence differences in standardized target regions within the chloroplast genome to distinguish among species.

Höltken et al. (2012) have developed and applied a set molecular markers to identify different high-value timber species in the family of Meliaceae including *Swietenia* sp. (listed on CITES appendix II), *Khaya*, *Entandrophragma*, and *Carapa* sp. (legal trade timbers). This study demonstrates the process of developing DNA markers for identification purposes. A detailed sequence analysis of several noncoding cpDNA regions resulted in an assay of seven genus-specific SNP (single nucleotide polymorphism) markers. Tools have been designed that could be applied with low-cost equipment on the basis of PCR-RFLPs without the need for sequencing or capillary electrophoresis techniques. In addition, the application of the method to wood material with degraded DNA of low overall quantity is highlighted.

In another approach Duminil et al. (2006, 2012) used a set of gene markers to assign trees of the genus *Carapa* in Latin America and in Africa to different species. Using a Bayesian approach, >90 % of the samples of *Carapa* in the Neotropics could be assigned to one of two distinct clusters

corresponding to previously described species, making it possible to estimate the genetic structure of each species and to identify cases of introgression. Duminil et al. argued that this blind procedure represents a first-choice rather than a fallback option whenever related taxa are investigated.

Control of the Geographic Origin of Timber

The control of the geographic origin in timber is an important issue as well. The falsification of the country of origin is another well-documented area of illegality in the trade in tropical timber. This occurs at the point of import for timber that is in international trade and usually involves the production of false paperwork such as phytosanitary certificates, invoices, and certificates of origin. An actual example is the ban of the EU and the USA on teak from Burma. Another common problem of illegal logging on smaller spatial scales is the false declaration of timber that has been logged outside a registered concession, or within a protected area. On this scale certified forest companies might have an economic interest to apply genetic fingerprints to prove their efforts of sustainable forest management.

In natural forests usually a genetic structure at local and regional spatial scales can be observed. In temperate forests as well as in tropical forests, the glacial periods changed the vegetation drastically. In the temperate zone large areas were covered by ice and were free of any vegetation, and in the tropics former rain forests were transformed to dry savannas. After each glacial period trees recolonized their distribution area starting from different refugia. As a result of this recolonization in many cases, a clear genetic differentiation can be identified between tree populations from different regions. The extent of genetic differences depends on the recolonization routes and the genetic differences in these refugia. Chloroplast gene markers and nuclear microsatellites have been successfully used to elaborate reference data about this genetic differentiation (Petit et al. 1997; Caron et al. 2000; Dutech et al. 2000, 2003; Hardy et al. 2013). Limited pollen and seed dispersal are the main factors causing spatial genetic structure on smaller scales in natural tree populations (Degen et al. 2001, 2004; Hardy et al. 2006). The spatial resolution of a possible control of timber origin depends on local and regional genetic differences and on the number of sampled populations used to generate a genetic reference database (Degen et al. 2001; Cavers et al. 2005).

DNA tests to assign and control the geographic origin of timber have been successfully applied on different spatial scales: Degen et al. (2013) have developed a genetic reference database to check the country of origin for *Swietenia macrophylla* in Latin America. Jolivet and Degen (2012) created a genetic reference database that was used to check a 70,000 ha forest concession of origin for sapeli in Cameroon. And Lowe et al. (2010) used DNA fingerprints to verify the chain of custody of individual merbau trees.

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